IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE APPLICANT	: David S. F. Young et al
INVENTION	: **CANCEROUS DISEASE MODIFYING ANTIBODIES**
SERIAL NUMBER	: 10/603,000
FILING DATE	: June 23, 2003
EXAMINER	: David J. Blanchard
GROUP ART UNIT	: 1643
OUR FILE NO.	: 2056.020

Commissioner for Patents
P. O. Box 1450
Alexandria, VA 22313-1450

DECLARATION UNDER 37 CFR § 1.132

I, David S. Young, do hereby declare as follows:

1. I am an inventor in the application entitled "**CANCEROUS DISEASE MODIFYING ANTIBODIES**", having U.S. Application Serial No. 10/603,000, filed June 23, 2003.

2. This declaration, including the attached data, is submitted in order to evidence the propriety of the claim terminology, and to evidence that both the novel antibodies and their antigen binding fragments, as instantly claimed in this application, are effective to induce cellular cytotoxicity in cancer cells absent any required interaction with the Fc region.

3. Pursuant to a teleconference on October 6, 2006 between Examiner David Blanchard and Ferris Lander (Applicants' representative) the Examiner's objections/rejections of record were thoroughly discussed, particularly regarding the terminology used in the claims and the sufficiency of the disclosure in providing an appropriate basis for the claim terminology and methodology.

During the teleconference, it was indicated that the presently claimed antibodies and antigen binding fragments thereof in the absence of the Fc region, are cytotoxic to cancer cells as shown by cytotoxicity assays conducted in the absence of complement.

Particularly, in the Office Action mailed on April 25, 2006, the Examiner notes that the hybridoma produce complete immunoglobulins and not antigen binding fragments, and cites Presta et al as evidence that cytotoxicity is mediated through ADCC and CDC via the Fc, and associated Fc receptors. It is well established (Paul et al, Fundamental Immunology, 2003, Lippincott) that the Fab (fragment of antigen binding) which contain variable and constant regions, can be produced by various cellular mechanisms to "generate nearly an infinite variety of molecules designed to recognize antigen" (p51). Moreover, the Fc domain can recruit cells and serum components to generate antibody

mediated cytotoxicity (ADCC, CDC, and CMC) through a variety of mechanisms, as taught by Presta et al, and Paul et al, among others.

However, the cytotoxicity mediated through the Fc region requires the presence of effector cells and their corresponding receptors, or proteins eg NK cells, complement, and T-cells, respectively. In the absence of these effector mechanisms, the Fc portion of an antibody is inert. The Fc portion of an antibody may confer properties that affect the pharmacokinetics of an antibody *in vivo*, but *in vitro* this is not operative.

The cytotoxicity assays under which we test the antibodies do not have any of the effector mechanisms present, and are carried out *in vitro*. These assays do not have effector cells (NK, Macrophages, or T-cells) or complement present. Since these assays are completely defined by what is added together, each component can be characterized. The assays used herein contain only target cells, media and sera. The target cells do not have effector functions since they are cancer cells or fibroblasts. Without exogenous cells which have effector function properties there is no cellular elements that have this function. The media does not contain complement or any cells. The sera used to support the growth of the target

cells do not have complement activity as disclosed by the vendors. Furthermore, in our own labs we have verified the absence of complement activity in the sera used. Therefore, our work evidences the fact that the effects of the antibodies are due entirely to the effects of the antigen binding which is mediated through the Fab. Effectively, the target cells are seeing and interacting with only the Fab, since they do not have receptors for the Fc. Although, the hybridoma is secreting complete immunoglobulin which was tested with the target cells, the only part of the immunoglobulin that interacts with the cells are the Fab, which act as antigen binding fragments.

With respect to the instantly claimed antibodies and antigen binding fragments, the application, as filed, has demonstrated cellular cytotoxicity. As pointed out above, and as herein confirmed via objective evidence, this effect was entirely due to binding by the Fab to the tumor cells.

Ample evidence exists in the art of antibodies mediating cytotoxicity due to direct binding of the antibody to the target antigen independent of effector mechanisms recruited by the Fc. The best evidence for this is *in vitro* experiments which do not have supplemental cells, or complement (to formally exclude those mechanisms). These types of experiments have been carried

out with complete immunoglobulin, or with antigen binding fragments such as F(ab)'2 fragments. In these types of experiments, antibodies or antigen binding fragments can directly induce apoptosis of target cells such as in the case of anti-Her2 and anti-EGFR antibodies, both of which have antibodies that are approved by the US FDA for marketing in cancer therapy.

4. The following experiments were performed in support of this Declaration:

Fetal bovine serum (FBS) used in *in vitro* assays may play a role in CDC antibody activity. In order to determine if this is the case we designed an experiment in which we investigated whether the 10% FBS routinely used in cytotoxicity assays would contain sufficient complement to activate cell lysis in the presence of the anti-HLA antibody W6/32 (CLHLA-01AP, Cedarlane Laboratories Limited, Hornby, ON, Canada).

Initial experiments were carried out, which demonstrated that W6/32, in the presence of 10% rabbit complement, could efficiently lyse the breast cancer cell line MDA-MB-231 (Figure 1). In these experiments, MDA-MB-231 cells (ATCC, Manassas, VA) were plated and allowed to adhere for 3 days. They were then treated with antibody for 1 hr, and then complement (10% final concentration;

Cat# CL3221, Cedarlane Laboratories Limited, Hornby, Ontario) was added for 3 hrs. Complement activity was assessed by measuring the amount of lactate dehydrogenase remaining on the plate after washing off cell fragments and debris. LDH was measured using an assay kit (Cytotox-96™, Promega, Madison, WI) The percent lysis was calculated as the amount of LDH remaining (an indicator of the number of intact cells) compared to the untreated controls. Complement in the absence of antibody resulted in minimal cytolysis in this cell line.

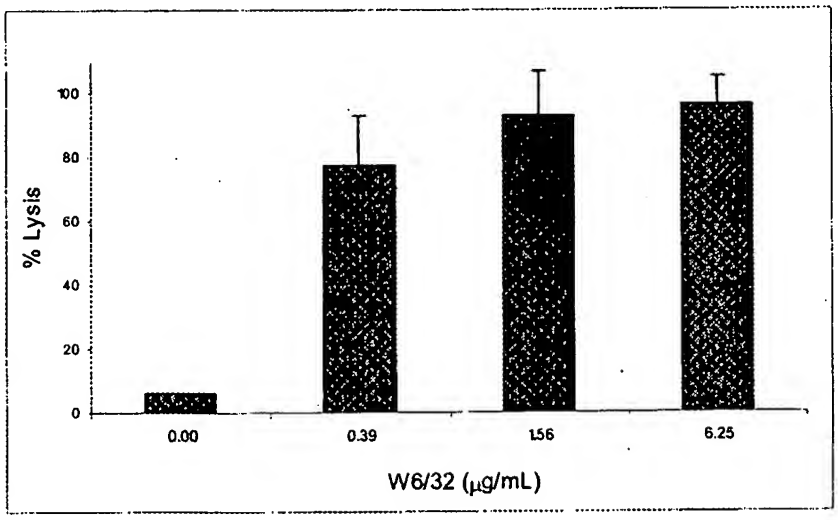

Figure 1. Effect of increasing concentrations ofW6/32, in the presence of 10% rabbit complement, on the lysis of human breast cancer MDA-MB-231 cells.

Experiments were then carried out to determine if the cytotoxic effect of antibodies incubated with cells in the presence of 10% FBS could be due to the presence of

complement in the serum. Cells were incubated with and without W6/32 in the presence of 10% FBS (Hyclone, Logan, Utah) only, 0.5% FBS only (essentially serum-free) and 10% rabbit complement (positive control). If there was sufficient complement in the serum to induce complement mediated lysis, one would expect to see an effect of W6/32 in the 10% FBS samples and no effect in the 0.5% FBS samples.

In this experiment, complement activity was measured using the ACEA RT-CES system. This system uses electrodes implanted in each well to measure the impedance across a cell monolayer. Changes in impedance, which can be monitored continually, are caused by changes in cell shape and number. MDA-MB-231 cells were plated at a density of 40,000 cells per well, and allowed to attach and grow for 24 hr until the cells reached a cell index of 1. Media was aspirated, and then replaced with media containing 10% FBS, 0.5% FBS, W6/32 (6.25 □g/mL) or 10% rabbit complement as indicated. Cells were monitored for an additional 6 hrs.

Figure 2 demonstrates that there were no differences in the curves from wells treated with 10% FBS, 0.5% FBS, or these media in the presence of W6/32. By contrast, a large shift was seen in the curves from wells treated with 10% complement and 10% complement in the presence of W6/32.

These latter 2 curves are also very different from each other, as would be expected based on results shown in Figure 1. These results indicate that results for 10% serum are different from those of complement-supplemented media, and are similar to those for serum-free media.

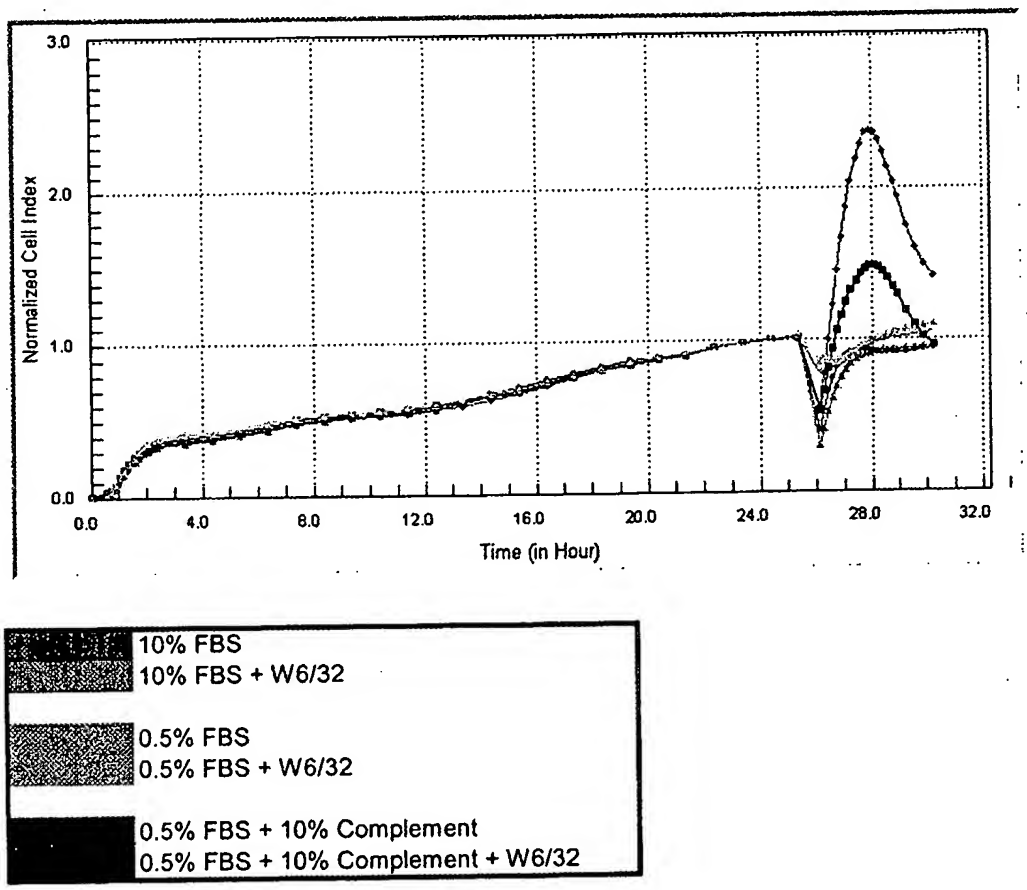

Figure 2. Impedance plot of MDA-MB-231 cells treated with FBS, W6/32 and 10% complement at 25 hr as indicated in the legend. Cell index was normalized at 25 hrs.

In conclusion, the FBS used in our labs does not contain bovine complement effective to initiate a CDC response in the presence of an antibody that has strong CDC

activity. These results confirm that any effects of ARIUS functional antibodies, assessed in the presence of 10% FBS, should be due to the antibody alone.

5. Accordingly, the antibodies and cellular toxicity inducing antigen binding fragments thereof, as instantly claimed, are clearly effective in mediating cellular cytotoxicity absent Fc interaction.

The undersigned declares that all statements made herein of his own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the Application or any patent issuing thereon.

Oct 6, 2006
Date

Dr. David S. Young